APPENDIX A

DECLARATION UNDER 37 C.F.R. 1.132 BY DR. MASAAKI MORI

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Masaaki MORI et al. Docket No. 506001 (46342)

Serial No. 09/869,540 Examiner: Jiang, Doug

Filed: June 27,2001 Group Art Unit: 1646

For: SCREENING METHOD

DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

....

Sir:

I, Masaaki MORI, the undersigned, a citizen of Japan residing at 3-8-5, Kasuga, Tsukuba-shi, Ibaraki 305-0821, JAPAN do hereby declare:

That I am an employee of the Assignee of the above-identified application;

That I graduated from University of Tokyo with the degree of Bachelor of Agriculture in March, 1978, and received a Ph. D. from University of Tokyo for a thesis entitled "Chemical studies on the sex pheromones in Streptococcus faecalis" in March, 1985;

That I was a postdoctoral fellow at University of Tokyo as a recipient of Japan Society for the Promotion of Science research fellowships for young scientists from April, 1985 to March, 1987;

That I have been employed by Takeda Chemical Industries, Ltd., Osaka, Japan, since April, 1987, and have been engaged in pharmaceutical research of said company,

That I was a visiting scientist in U.S.-Japan Biomedical Research Laboratories, Tulane University School of Medicine from May, 1988 to July, 1989;

That I am a member of the Japan Society for Bioscience, Biothechnology, and Agrochemistry, the Japanese Biochemical Society, the Japanese Peptide Society, and the Japanese Pharmacological Society, and published with other research workers, a number of reports on scientific studies, among others, including

(1) Purification of acidic fibroblast growth factor from bovine omentum, Biochem. Biophys. Res. Commun., 161, 169-175 (1989), Tetsuya Ohtaki, Kaori Wakamatsu, Masaaki Mori, Yoshihiro Ishibashi, Tadashi Yasuhara;

(2) Oxytocin is the major prolactin releasing factor in the posterior pituitary, Endocrinology, 126, 1009-1013 (1990), Masaaki Mori, Sandor Vigh, Atsuro Miyata, Tadashi Yoshihara, Shusaku Oka, Akira Arimura;

(3) Isolation and identification of hemin as an endogenous Na^+/K^+-ATPase inhibitor from porcine blood cells, Biochem. Biophys. Res. Commun., 178, 95-103 (1991), Tadashi Yasuhara, Masaaki Mori, Kaori Wakamatsu, Kazuki Kubo;

(4) Isolation and identification of melanin-concentrating hormone as the endogenous ligand of the SLC-1 receptor, Biochem. Biophys. Res. Commun., 261, 622-626 (1999), Yukio Shimomura, Masaaki Mori, Tsukasa Sugo, Yoshihiro Ishibashi, Michiko Abe, Tsutomu Kurokawa, Haruo Onda, Osamu Nishimura, Yasuhiro Sumino, Masahiko Fujino;

(5) Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14), Biochem. Biophys. Res. Commun., 265, 123-129 (1999), Masaaki Mori, Tsukasa Sugo, Michiko Abe, Yukio Shimomura, Mika Kurihara, Chieko Kitada, Kuniko Kikuchi, Yasushi Shintani, Tsutomu Kurokawa, Haruo Onda, Osamu Nishimura, Masahiko Fujino;

(6) Cloning of a novel G protein-coupled receptor, SLT, a subtype of the melanin-concentrating hormone receptor, Biochem. Biophys. Res. Commun., 283, 1013-1018 (2001), Masaaki Mori, Mioko Harada, Yasuko Terao, Tsukasa Sugo, Takuya Watanabe, Yukio Shimomura, Michiko Abe, Yasushi Shintani, Haruo Onda, Osamu Nishimura, Masahiko Fujino;

(7) T-226296: a novel, orally active and selective melanin-concentrating hormone receptor antagonist, Eur. J. Pharmacol., 438, 129-135 (2002), Shiro Takekawa,

Asano Asami, Yuji Ishihara, Jun Terauchi, Kaneyoshi Kato, Yukio Shimomura, Masaaki Mori, Hitomi Murakoshi, Koki Kato, Nobuhiro Suzuki, Osamu Nishimura, Masahiko Fujino;

(8) Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7, J. Biol. Chem., 277, 34010-34016 (2002), Ryo Fujii, Hiromi Yoshida, Shoji Fukusumi, Yugo Habata, Masaki Hosoya, Yuji Kawamata, Takahiko Yano, Shuji Hinuma, Chieko Kitada, Taiji Asami, Masaaki Mori, Yukio Fujisawa, Masahiko Fujino;

(9) Identification of neuropeptide W as the endogenous ligand for orphan G-protein-coupled receptors, GPR7 and GPR8, J. Biol. Chem., 277, 35826-35832 (2002), Yukio Shimomura, Mioko Harada, Mika Goto, Tsukasa Sugo, Yoshio Matsumoto, Michiko Abe, Takuya Watanabe, Taiji Asami, Chieko Kitada, Masaaki Mori, Haruo Onda, Masahiko Fujino;

(10) A role for neuropeptide W in the regulation of feeding behavior, Endocrinology, 144, 4729-4733 (2003), Muhtashan S. Mondal, Hideki Yamaguchi, Yukari Date, Takuya Shimbara, Koji Toshinai, Yukio Shimomura, Masaaki Mori, Masamitsu Nakazato;

(11) Identification of urotensin II-related peptide as the urotensin II-immunoreactive molecule in the rat brain, Biochem. Biophys. Res. Commun., 310, 860-868 (2003), Tsukasa Sugo, Yuko Murakami, Yukio Shimomura, Mioko Harada, Michiko Abe, Yoshihiro Ishibashi, Chieko Kitada, Nobuyuki Miyajima, Nobuhiro Suzuki, Masaaki Mori, Masahiko Fujino; and

(12) Intracerebroventricular administration of urotensin II promotes anxiogenic-like behaviors in rodents, Neurosci. Lett., 358, 99-102 (2004), Yoshio Matsumoto, Michiko Abe, Takuya Watanabe, Yuka Adachi, Takahiko Yano, Hideki Takahashi, Tsukasa Sugo, Masaaki Mori, Chieko Kitada, Tsutomu Kurokawa, Masahiko Fujino;

That I am one of the inventors of the above-identified patent application (hereinafter referred to as "this application"); and

That I have read the Final Office Action mailed March 9, 2004, including the grounds or remarks provided by the Examiner as to why Claims 1, 2, and 12-14 were considered unpatentable over the cited art.

That this declaration is being submitted to address certain conclusions reached by the Examiner as to the teachings and disclosure of Salon (U.S. Patent 6,221,616) and Ames (U.S. Patent Publication 2002/0038007), each in view of Maratos-Flier (U.S. Patent 849,708) and Bolton (*Biochem. J.*, 1973, 133:529-539), cited in support of the rejection of the claims.

That for at least the reasons set forth below, the method for screening a compound that alters the binding between [^{125}I][N-(3-(4-hydrxoy-3-iodophenyl) propionyl)-met^{4}]-MCH(4-19) and SLC-1 provided by the invention is not anticipated or obvious in view of the teachings of Ames in view of Maratos-Flier and Bolton or in view of the teachings of Salon in view of Maratos-Flier and Bolton.

That the following experiments were carried out by myself or under my direction:

EXPERIMENT

Assay for the agonist activity of MCH, MCH(2-19), MCH(3-19), MCH(4-19) and MCH(5-19) derivatized with a non-isotope Bolton-Hunter reagent (hereinafter referred to as "BH reagent"), using the GTPγS binding assay

The agonist activity of the non-isotope BH reagent-derivatized MCH, MCH(2-19), MCH(3-19), MCH(4-19) and MCH(5-19) obtained in Example 18 was assayed using the GTPγS binding assay as shown in Example 22 of this application.

The derivatized MCH, MCH(2-19), MCH(3-19), MCH(4-19) and MCH(5-19) increased dose-dependently the amount of [^{35}S]-guanosine 5'-(γ-thio)triphosphate bound to the human SLC-1-expression CHO cell membrane fraction, confirming that various MCHs derivatized with a non-isotope Bolton-Hunter reagent exhibit the agonist activity as shown in Fig. 8 of this application.

The results of Example 22 can be represented by EC_{50} values as follows.

	EC_{50} (nM)
MCH	0.55
BH-MCH	1.95
BH-MCH(2-19)	2.64
BH-MCH(3-19)	1.27
BH-MCH(4-19)	0.43
BH-MCH(5-19)	1.95

As shown above, when full-length MCH was derivatized using the BH reagent to prepare BH-MCH, its agonist activity was reduced by 3.5 fold compared to un-derivatized MCH. Surprisingly, it was found that the agonist activity of BH-MCH(4-19) exhibited 4.5 fold activity compared to the BH-MCH, and that its agonist activity is higher than the full-length MCH. Also, it was unexpectedly found that the agonist activity of BH-MCH(4-19) was 6.1 times higher than BH-MCH(2-19); 3.0 times higher than BH-MCH(3-19); and 4.5 times higher than BH-MCH(5-19) although the difference between them resides only in absence or presence of one or two amino acid residues.

In addition, it was confirmed in Example 23 of this application that the [^{125}I]-labeled BH-MCH(4-19) exhibited sufficient SLC-1 binding specificity to extent that the binding inhibition assay can be efficiently carried out (See Example 23 and Fig. 9).

Therefore, to my best knowledge, I believe that the agonist activity of BH-MCH(4-19), i.e., [N-(3-(4-Hydroxy-3-iodophenyl)propionyl)-Met^4]-MCH(4-19) was unexpected, and thus use of the radiolabeled BH-MCH(4-19), i.e., [^{125}I]-[N-(3-(4-Hydroxy-3-iodophenyl)propionyl)-Met^4]-MCH(4-19) for screening a compound that alters the binding property of MCH and SLC-1

was considered unobvious.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 26 day of April, 2004.

Masaaki Mori

Masaaki MORI, PhD